Genotype–phenotype correlations in laminopathies: how does fate translate?

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Abstract

A-type laminopathies are a group of diseases resulting from mutations in the intermediate filament proteins lamin A and C (both encoded by the LMNA gene), but for which the pathogenic mechanisms are little understood. In some laminopathies, there is a good correlation between the presence of a specific LMNA mutation and the disease diagnosed. In others however, many different mutations can give rise to the same clinical condition, even though the mutations may be distributed throughout one, or more, of the three functionally distinct protein domains of lamin A/C. Conversely, certain mutations can cause multiple laminopathies, with related patients carrying an identical mutation even having separate diseases, often affecting different tissues. Therefore clarifying genotype–phenotype links may provide important insights into both disease penetrance and mechanism. In the present paper, we review recent developments in genotype–phenotype correlations in laminopathies and discuss the factors that could influence pathology.

Introduction

Mutations in the LMNA gene cause a variety of diseases collectively called ‘laminopathies’. To date, more than 340 unique LMNA mutations (http://www.umd.be) [1] are known that cause 16 different diseases including A-EDMD (autosomal Emery–Dreifuss muscular dystrophy), DCM (dilated cardiomyopathy), LGMD1B (limb-girdle muscular dystrophy 1B), L-CMD (LMNA-related congenital muscular dystrophy), FPLD2 (familial partial lipodystrophy 2), HGPS (Hutchinson–Gilford progeria syndrome), atypical WRN (Werner syndrome), MAD (mandibuloacral dysplasia) and CMT2B (Charcot–Marie–Tooth disorder type 2B).

The LMNA gene is alternatively spliced to produce the type V intermediate filament proteins lamin A, C, C2 and delt10. Together with B-type lamins, lamin A and C are the major components of the nuclear lamina (a fibrous proteinaceous meshwork underlying the nuclear envelope). The nuclear lamina functions to maintain both nuclear and cellular architecture and plays a role in chromatin organization and gene expression [2]. The presence of mutant forms of lamin A/C is thought to perturb some, or all, of these functions of the nuclear lamina, but it is generally unclear how specific mutations result in a particular laminopathy. Phenotypic clustering, the systematic correlation of phenotype with genotype, is becoming increasingly important to understand underlying mechanisms of monogenic diseases [3–5]. Here, we review the correlation of LMNA mutation with clinical diagnosis, to explore genotype–phenotype links within laminopathies (Figure 1).

Laminopathies with a more consistent genotype-phenotype link

To date, mutations distributed throughout the gene and affecting almost 20% of the coding sequence of LMNA have been reported from more than 1000 patients, and arise mainly from missense or frameshift mutations (http://www.umd.be) [1]. In some laminopathies such as HGPS, ‘hot-spot’ or founder mutations result in a similar phenotype. HGPS is almost always caused by the de novo base mutation c.1824C>T/p.G608X, which results in a cryptic splice donor site in the lamin A-specific exon 11, and deletion of the remaining 50 amino acids of the C-terminus [6]. The resulting truncated protein (termed progerin) retains the CAAAX box but lacks the endoproteolytic cleavage site, so it cannot be processed by Zmpste24, and is incorporated into the nuclear lamina carrying the C-terminal farnesyl group. Two unrelated patients with severe forms of HGPS have recently been reported carrying two new mutations (c.1968+1G>A and c.1821G>A), but both cause a frequent use of the splice donor site that is active in typical HGPS patients [7]. Other laminopathies also appear to arise from a common LMNA mutation, such as WRN (R133L), CMT2B (R298C) and MAD (R527H) (although R133L also causes FPLD2, and R527H additionally results in A-EDMD) (Figure 1) [8]. Although the number of patients developing HGPS, WRN, CMT2B or MAD is small, it is likely that the presence of a particular mutation will have a predictive value on the
Figure 1 | Distribution of lamin A mutations and their related laminopathies
(a) Schematic diagram of the \textit{LMNA} gene, with exons encoding their respective protein domains colour coded and the lamin A splice site indicated in red above. HGPS mutations causing a new splice donor site are indicated in black boxes. (b) The lamin A protein with mutations indicated that result in A-EDMD (blue), DCM (green), LGMD1B (black), L-CMD (gold), FPLD2 (red) and CMT2B (plum). '†' indicates that the same amino acid change causes different laminopathies. *R133L also causes WRN, *S143F additionally results in HGPS, *R527H causes MAD too and *R644C also gives rise to a range of other disorders [26–29]. †Patients carrying R527C develop either a severe form of MAD and/or progeria [52,53]. Mutation/laminopathy correlations are from [1,9]. Interacting proteins and their corresponding binding regions on lamin A are indicated below [54].

clinical diagnosis and that the pathogenic mechanism will be similar for a given disorder (Figure 2a). Importantly, these disorders are characterized by systemic effects, with many tissues affected, unlike the more common laminopathies.

**Laminopathies with an inconsistent genotype–phenotype link**

By contrast, laminopathies with striated muscle involvement (A-EDMD, LGMD1B, L-CMD and DCM), together accounting for $\sim$60% of all laminopathies, along with those affecting adipose tissue (FPLD2), are caused by a large number of different mutations (Figure 1). A-EDMD (blue in Figure 1) and L-CMD (gold in Figure 1) mutations are distributed throughout the gene, whereas LGMD1B (black in Figure 1) mutations tend to cluster in both the Ig-like fold and coil 2 [1,9]. DCM (green in Figure 1) patients tend to carry mutations throughout the rod domain and can share clinical characteristics with A-EDMD patients, where an isolated cardiac involvement has been described. For FPLD2, there is an apparent ‘hot spot’ for mutations in the Ig-like fold where 75% of all mutations occur. The remaining 25% are distributed throughout the protein and are distinct from those causing A-EDMD (Figure 1). Interestingly, muscle involvement has not been observed in FPLD2 patients. The C-terminal domain of lamin A binds SREBP1 (sterol-regulatory-element-binding protein 1) and SREBP2, which play a key role in adipocyte differentiation and cholesterol biosynthesis respectively [10]. Thus certain mutations may disrupt lamin A–SREBP1/2 interactions to specifically affect adipocyte function.

**Phenotypic clustering using mutation type**

Bonne et al. [11] first attempted a genotype–phenotype correlation by mutation type in \textit{LMNA}. Of the 53 patients analysed, all 12 with isolated heart involvement carried a nonsense mutation in Q6X of the head domain. In the remaining 41 patients with muscle weakness however, attempts to correlate disease severity with the protein domain affected by the missense mutations proved inconclusive. Our own analysis of \textit{LMNA} patients (http://www.interfil.org)
the domain surface without affecting structural integrity: with R482W, for example, not changing the crystal structure of the mutant protein [13–15]. However, tetrameric aggregates of mutant molecules were found that did not occur in the original structure, such that R482W or R482Q might cause an allosteric effect, allowing the repositioning of the C-terminal β-strand g′, leading to a novel aggregation state and so possibly contributing to disease [13]. A similar phenomenon occurs with the F12L mutation in porphobilinogen synthase, which initiates a transition of the aggregation state, significantly changing the enzyme kinetics [16]. Certain mutations, therefore, could have a common mechanism to cause a particular laminopathy, but it is less clear how mutations located in functionally distinct protein domains could operate by the same disease mechanism (Figures 1 and 2b).

**Differential effects of LMNA mutations in post-mitotic cells compared with dividing cells**

An LMNA mutation causing A-EDMD (R453W) perturbs myogenic differentiation, while an FPLD2-causing mutation (R482W) does not, again suggesting that certain mutations can have tissue-specific effects [17]. Many LMNA mutations probably have common effects, including compromised nuclear integrity and transcriptional regulation, but some may also have additional actions. Particular mutant lamin A variants delay cell cycle progression by prolonging S-phase [18], and some also hinder the exit from cell cycle and/or the nuclear rearrangements, required for myogenic differentiation in immortalized cell lines [17,19]. The functional cells of cardiac muscle, skeletal muscle and adipose tissue are post-mitotic, but skeletal muscle retains a well-characterized stem cell compartment, responsible for homoeostatic myonuclear turnover, hypertrophy and repair [20]. Thus skeletal muscle may additionally be vulnerable to mutations that also affect cell cycle and/or differentiation of myogenic stem cells. Such a mutation, therefore, may not only influence the onset and severity of A-EDMD [21] or produce L-CMD [9], but even influence which tissue is affected and so the type of laminopathy caused.

**Lamin A or lamin C or both?**

Since LMNA is alternatively spliced [22], another way to classify mutations is whether they affect both lamin A and lamin C or just one protein. Of the 12 exons in LMNA, exons 11 and 12 are specific to lamin A, while alternative splicing at codon 566 in exon 10 gives lamin C, a unique exon coding for six amino acid residues (Figure 1). As expected given its size, only two mutations, R571S causing DCM [23] and R571C causing muscular dystrophy with axonal neuropathy [24], have been associated with the lamin C-specific tail. Exon 12 encodes eight amino acids that are cleaved during post-translational modification of prelamin A, so unless mutations affect prelamin A processing, they would not be expected to affect lamin A function. Interestingly, few mutations occur in lamin A-specific exon 11 and those that do are less

**Structural changes of mutant lamin A in A-EDMD, but not in FPLD2**

Mutations in the Ig-like fold provide evidence of a genotype-phenotype correlation [13,14]. This globular region in the C-terminal domain harbours both A-EDMD (e.g. R453W and R572P) and FPLD2 (e.g. R482W, R482Q and K486N), causing mutations in close proximity (Figure 1). Mutations causing laminopathies with striated muscle involvement occur at positions that play a critical role in the structural stability of the C-terminal domain. Conversely, those that lead to FPLD2 result from a lost positive charge on
associated with skeletal muscle involvement: for example, of 18 mutations in exon 11 [1], 56% (10/18) cause DCM and 33% (6/18) result in FPLD2, with only one each causing A-EDMD or LGMD1B (J. Scharner, V. Gnocchi, J.A. Ellis and P.S. Zammit, unpublished work). It is worth noting that as HGPS predominately, but not exclusively, targets lamin A and generates the most severe laminopathy, it has been interpreted as being the more important protein. However, the apparent normal phenotype of the Lmna<sup>LCO</sup>/LCO<sup>L</sup> mouse, where lamin C is the only A-type lamin present, suggests that both lamin A and the other isoforms are dispensable [25].

**Effect of modifying genes and SNPs (single nucleotide polymorphisms) on phenotypic variability in laminopathies**

Patients carrying, for example, the R644C mutation have a range of clinical conditions including DCM, LGMD2B, atypical HGPS, lipodystrophy etc. [26–29]. This extreme phenotypic variability indicates that genetic background contributes to the disease diagnosed [29]. Furthermore, a single mutation can result in DCM either with or without A-EDMD. This provides evidence of epistasis, i.e. effects of the mutated LMNA gene are altered by one or more other genes, the so-called modifying genes (Figure 2c). There are, indeed, rare examples of where an additional mutation in either desmin or emerin results in a more severe disease than might be expected from the LMNA mutation alone [30].

Where patients carry two different pathogenic LMNA mutations, it is not unexpected that they develop a more severe phenotype [31]. However, LMNA also contains SNPs with no apparent pathological phenotype: of 40 on the Leiden Open Variation Database (www.dmd.nl/lmna_seqvar.html), 75% (30/40) are silent mutations, and the rest are missense mutations affecting the head (one), central rod (three) or tail (six) domains (J. Scharner, V. Gnocchi, J.A. Ellis and P.S. Zammit, unpublished work). Depending on the context however, these mutations can cause disease: T528M or M540T alone appear non-pathogenic, but when inherited together, they result in an apparently typical HGPS but without prelamin A accumulation [32]. Certainly, a particular LMNA pathogenic mutation/SNP combination can increase the penetrance of a phenotype, which offers an explanation for intra- and inter-familiar variations [33], but may also alter the clinical condition diagnosed: while the S583L mutation normally causes FPLD2, when present with T528M it results in FPLD1 [34]. Modifying genes/SNPs are likely to explain inconclusive attempts to associate mutation with laminopathy (Figure 2c), where, for example, a single mutation has been reported to express phenotypic variability, such as with R60G, Y267C, R377H/L and R644C (Figure 1).

**Mouse models of laminopathies**

Modelling diseases in mice is a powerful technology to explore genotype–phenotype correlations, disease mechanisms and potential therapies. The majority of laminopathies arise from missense and frameshift mutations, but two nonsense mutations have been reported: patients heterozygous for Y259X have LGMD1B [35], but those heterozygous for Q6X develop DCM [36,37] and mice heterozygous for the targeted Lmna null allele also develop a DCM phenotype [38]. The only LMNA–null homozygous subject identified carried Y259X and died at birth, and Lmna<sup>−/−</sup> mice are characterized by postnatal growth retardation, muscular dystrophy, rapidly progressive DCM and death by 4–8 weeks of age [39,40].

In humans, both the L530P and H222P mutations cause A-EDMD. The H222P mouse recapitulates features of A-EDMD [41], while L530P causes a phenotype akin to HGPS, possibly due to the unintentional inclusion of an additional splicing defect in the C-terminus [42]. The N195K mutation causes DCM in humans and a DCM-like phenotype in mice [43]. Importantly, the L530P, H222P and N195K mouse models only show a phenotype when homozygous for the mutant alleles, in contrast with the heterozygous state in patients. DCM with A-EDMD resulting from the M371K mutation also causes a heart phenotype in transgenic mice when under a heart-specific promoter [44]. Collectively, these mutant mice illustrate that aspects of human laminopathies can be successfully modelled in mice, with all the attendant advantages over experimenting on rare patient samples, but again emphasizes that genetic background exerts an influence on the penetrance of the condition, whether in mice or humans.

**Conclusions and perspectives**

It must be remembered that laminopathies are rare diseases, and in many cases there are very few patients with a particular mutation(s). That said, what can be concluded about genotype–phenotype correlations? In laminopathies such as HGPS, there is a high degree of consistency in the underlying mutation between patients, meaning that the disease mechanism is likely to be common to that particular disorder. There is also a degree of genotype–phenotype correlation in FPLD2 for example, where many mutations are in the Ig-like fold and so could have a common disease mechanism, although the rest give little clue as to why they cause this condition. For others however, including DCM, A-EDMD and LGMD1B, there is a much weaker correlation between mutation and disease (Figure 2). A-EDMD and FPLD2 may differ owing to the degree to which a particular mutation affects lamin A/C structure, but this does not, for example, explain how different amino acid substitutions lead to a specific phenotype. It should also be remembered that lamins A and C are distinct proteins and mutations in the common region of LMNA would generate two distinct mutated proteins that could function differently to cause disease and so need to be treated as individual entities [45].

Where a more severe or unexpected phenotype is found with a particular LMNA mutation, a number of factors may be contributing. Whether a mutation also affects cell cycle and differentiation, in addition to nuclear structure, chromosome
organization or transcriptional regulation, could certainly contribute to disease onset and severity, but maybe even the laminopathy diagnosed. The emerging evidence of modifying genes and SNPs affecting laminopathies means that, in future, it would be helpful if clinical data were to include the LMNA-specific SNPs, to determine if disease severity/diagnosis can be linked to certain mutation/SNP combinations. Screening LMNA for SNPs is also being used in non-laminopathies with overlapping phenotypes, to determine whether there is any correlation with disease severity [46]. Ultimately, genome-wide sequencing would be needed to check for mutations in all regulatory regions of LMNA and fully explore the correlation with modifying genes. Finally, EDMD is divided into A-EDMD, and X-linked EDMD caused by mutations in EMD, but together these account for only ~50% [47] of clinically diagnosed EDMD. Assuming that LMNA mutations/SNPs have not been overlooked, mutations in other genes may also result in disorders with a similar phenotype [48]. Indeed, it was recently shown that mutations in LAP2α can cause a condition similar to DCM [49], whereas mutations in SYNE1 or FHL1 produce an EDMD-like phenotype [50,51].

Funding

J.S. is supported by a Ph.D. studentship funded by the Biomedical and Health School, King’s College London, and Y.F.G. is funded by The Medical Research Council [grant number G0700307]. The P.S.Z. laboratory is supported by The Muscular Dystrophy Campaign, The Wellcome Trust and OPTISTEM (contract 223098), through the European Union 7th Framework Programme.

References

20 Zammit, P.S. (2008) All muscle satellite cells are equal, but are some more equal than others? J. Cell Sci. 121, 2975–2982

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Received 28 August 2009
doi:10.1042/BST0380257